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DNA supported graphene quantum dots for Ag ion sensing

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Abstract
The use of graphene quantum dots can be extended for bio-sensing and metal ion detection. Synergistic combination of graphene quantum dots (GQDs) with DNA leads to high performance Ag-ion detection system. The thoroughly characterized GQDs were found to have spherical morphology, with dimensions in the range of 5–10 nm. The atomic force microscopy studies proved that the synthesized GQDs were mostly comprised of two to four graphene layers. To make the system biocompatible, GQDs/NGQDs were combined with DNA. Two properties of DNA were exploited, capacity to provide nitrogen to GQDs; and to synergistically contribute to Ag⁺ detection. In addition to Ag⁺, the strong green photoluminescence (PL) of GQDs showed significant quenching, owing to the appearance of associated Förster resonance energy transfer processes. This led to high sensing efficiencies.

Supplementary material for this article is available online

Keywords: graphene quantum dot, DNA, PL sensor, FRET process, Ag-ion

1. Introduction
Graphene quantum dots (GQDs), having superior photo-stability, bio-compatibility and low toxicity are fast emerging as a fascinating alternative to conventional quantum dots (QDs) or organic dyes [1, 2]. Compared to carbon dots, GQDs can retain their 2D structure inside the dots. This stimulates of a large band gap, peculiar electronic structure and intriguing electrochemical, optoelectronic and/or luminescence properties [3–5]. The low cost, easy maintenance and high shelf life of GQDs is also being exploited in the field of energy and gas sensing applications [6, 7]. Ionic groups such as O⁻ and COO⁻, which form the planes and edges of the GQDs, can also catalyze electrostatic interactions with charged proteins and deoxyribonucleic acids (DNA). Consequently GQDs are now being investigated as a next generation metal-free fluorescent sensing nanostructure for biomedical applications ranging from detection, delivery, labeling to imaging [8, 9].

In recent times, incorporating hetero-atoms like N, B, P, etc. have led to GQDs with enticing semiconducting properties [10–12]. These GQDs show relatively higher positive charge density near the carbon atoms. This leads to tunable band gap, electronic conductivity, optoelectronic and photo-chemical properties [13, 14]. For example, N atom doped GQDs (N-GQDs) has exhibited enhanced quantum yield, visible emission, upconversion photoluminescence (PL) and fluorescence efficiency [15, 16]. The fluorescence in such a system has been attributed to the recombination of electron-hole pairs in localized electronic states. Hetero-atom doped GQDs have also been recently tested for deep tissue imaging and in vivo sensing [17, 18]. This paper discusses a new application for DNA functionalized GQD systems i.e., as an efficient biosensor for Ag⁺ detection.

Silver has a lot of advantages and few serious disadvantages, which makes its detection crucial for various industries. Ag-ions (Ag⁺), possessing intrinsic antibacterial
properties, have been traditionally used in medical science for treating infectious diseases [19]. It is also a proven biocide used for controlling micro-organisms [20]. But excessive or inadvertent use of silver can lead to diseases like diarrhea, skin infection, nervous or organ damage [21]. It has also been reported that Ag\(^+\) can cause cell lysis, inhibit cell growth, interact with biomolecules (protein, DNA, enzymes, etc.) and alter the functions/confirmation of biomolecules in a living cell and induce its death [22]. It can also act as a soft acid, which interacts with the soft base of a cell and induce its death.

It is shown that DNA functionalized GQD based systems have sensing detection limits in the range ∼0.02 to 30 nM, which is comparable or much lower than the toxicity level prescribed by the US Environmental Protection Agency [23]. It is observed that GQDs, prepared using a simple bottom-up approach, work as a fluorophore, while the Ag\(^+\) acts as a fluorescence quencher [24]. The observed energy transfer mechanism clearly indicates that GQDs can work as a high performance fluorescent sensing probe for label-free detection of Ag\(^+\), with high sensitivity and selectivity.

2. Experimental section

Citric acid (99%), sodium hydroxide pellets (97%), ammonium solution (25%) and silver nitrate were purchased from Merck Specialties Pvt. Ltd. (India). Calf thymus DNA (>90% pure) was purchased from GeNeiTM. The plasmid pET-28a was extracted through standard protocol mentioned in plasmid extraction and purification.

2.1. Synthesis of GQDs

The GQDs were prepared by the pyrolysis of citric acid using a simple bottom-up approach of synthesis [25]. To prepare GQDs, 2 g of citric acid was heated at 473 K for 10 min. The obtained colorless liquid was further heated for 25 min before it became an orange color solution, indicating the formation of GQDs. The obtained GQDs were added drop-wise into a 100 ml of 10 mg ml\(^{-1}\) NaOH solution under vigorous stirring and neutralized to pH 7.0.

2.2. Plasmid extraction and purification

DH5α E. coli cells, transformed with pET-28a, were grown on a Luria broth (LB) plate with kanamycin (50 mg L\(^{-1}\)). A single colony was taken to grow a starter culture in the LB medium containing kanamycin at 310 K for 6 h (OD660 = 0.8). The starter culture was diluted 1000 times into the LB medium containing kanamycin and grown at 310 K for 12 to 16 h, under vigorous shaking (280 rpm, OD660 = 1.8 for each batch). The cells were harvested by centrifugation at 6000 rpm for 15 min at 277 K. The pET-28a was isolated and purified from the harvested cells by QIAGEN Midi prep kit using a standard protocol. For visualization, the sample was analyzed through agarose gel electrophoresis by using O’GeneRuler 1 kb DNA Ladder (shown in figure S1 of the supplementary information, available online at stacks.iop.org/NANO/30/255501/mmedia). The purified plasmid was in a supercoiled form. It was then dissolved in 1x Tris/EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 8), filtered through a 0.22 \(\mu\)m Whatman syringe filter and stored at 253 K (see the supplementary information for detail).
3. Characterization techniques

The phase formation of the GQDs was confirmed by the analysis of x-ray diffraction data, collected using a PANalytical X’Pert diffractometer, with CuKα1 incident radiation (λ = 0.15406 nm) and in the 2θ range 10 to 80°. Transmission electron microscopy (TEM) (FEI-TECNAI G220S-Twin operated at 200 kV) and high resolution TEM (JEOL JEM-2100, operated at 80 kV) were used to investigate the morphologies of the prepared particles.

Atomic force microscopy (AFM) (Agilent Technologies, AZ, USA, Model-5500) imaging were used for particle surface studies of the synthesized GQDs. The cantilever, with spring constant of 1 N m\(^{-1}\), was used for scanning the sample surface. The sample was scanned in the tapping mode, with the help of an etched silicon tip (∼10 nm curvature radius), which was used to simultaneously record the height and amplitude.

The UV-Vis absorption spectra were recorded on spectrophotometer (AvantesStarline AvaSpec-ULS3648). The zeta potential was measured using the Horiba Scientific Nano Particle Analyzer SZ-100.

To collect the emission spectra, a Xenon lamp (450 watt) coupled to monochromator (Gemini 180) was used to measure the PL spectra. The PL data was recorded using a CCD detector (Hamamatsu), which was coupled to a monochromator (Horiba Jobin-Y von HR30). Laser light scattering measurements were undertaken in a Photocor Complex (USA) system comprising of a GaAs diode laser 35 mW, λ = 658.3 nm and log spaced 256 channel PMT. The static

<table>
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<td>GQDs</td>
<td>Quenching</td>
<td>30.0</td>
<td>1–20</td>
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<tr>
<td>NGQDs</td>
<td>Quenching</td>
<td>22.0</td>
<td>1–20</td>
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<tr>
<td>GQDs-pET28a</td>
<td>Quenching</td>
<td>0.96</td>
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<td>Quenching</td>
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<td></td>
<td></td>
<td>0.84</td>
<td>0.2–2</td>
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Figure 3. (a) PL Intensity profile for Ag\(^+\) detection of GQDs. (b) PL Intensity profile for Ag\(^+\) detection of NGQDs. (c) Stern–Volmer curve fitting of GQDs (d) Stern–Volmer curve fitting of NGQDs.

Table 1. Detection limits of Ag ion.
light scattering method, based on measurements of average scattering intensity, was performed in the angular range 30° to 140° in steps of 10°.

3.1. Quantum yield measurements

The quantum yield (Q) of the GQDs, in a water solution, was measured using the following relation [26].

$$ Q = Q_{std} \times \frac{M_s}{M_{std}} \times \frac{\eta_s^2}{\eta_{std}^2} $$

where $M_s$ and $\eta_s$ represent the slope of the linear plot and refractive index of the solvent for GQDs, respectively. $Q_{std}$, $M_{std}$ and $\eta_{std}$ denoted the quantum yield, slope and refractive index of the standard sample (methylene blue with quantum yield 0.52), respectively [27].

4. Results and discussions

The preliminary experiments such as: x-ray diffraction (XRD); Fourier-transform infrared spectroscopy (FTIR); and Raman and x-ray photoelectron spectroscopy (XPS), etc. were performed to confirm the formation of GQDs and are described in the supplementary information (see figure S2–S4). The size distribution of the GQDs, using TEM and AFM imaging, showed spherical QDs in the range 5–10 nm (see figures 1(a), (b)). The fringe patterns, observed in the high resolution TEM (shown in figures S5(a) and (b)), confirmed the formation of highly crystalline GQDs with interplanar spacing of 0.34 nm. This was similar to the (002) peak of the graphitic carbon [28]. In order to measure the third dimension i.e. height, AFM measurements were performed. Figure 1(b) depict the planer view of GQDs, deposited on a p-Si substrate. The height of the unmodified GQDs was found to be in the range of 1.0–3.0 nm [29]. The relative height difference between two layers of GQDs is reported to be 0.76 nm [30]. The AFM graphs, therefore suggest that the stabilized GQDs were comprised of one to four graphene layers.

The FTIR spectra (shown in figure 2) confirmed the presence of carboxyl (–COOH) and hydroxyl (–OH) groups in the synthesized GQDs. The characteristic absorption bands in the region 3000–3100 cm$^{-1}$ are generally attributed to the stretching vibrations of O–H and N–H [31]. Vibrations of the aromatic rings are linked with the peaks near 1450–1650 cm$^{-1}$. Strong absorptions observed in the range 1500–1800 cm$^{-1}$ are generally associated with the in-plane stretching of the nucleic bases, adenine-thymine (A-T), guanine-cytosine (G-C) and the characteristics vibrations of bonds C=O, C≡C, C≡N, and NH$_2$ groups [32]. Few additional weak absorption profiles, in the range 600–800 cm$^{-1}$, were also discernible. These are known to originate from the vibrations of aromatic and sugar-phosphate molecular backbone [33]. The FTIR spectra from NGQDs (shown in figure S3(b)) indicated slight modification of C=O bond vibration and, as expected, new peaks associated with the vibration of C–NH and C≡N bonds were observed [34, 35].

The GQDs have been proposed as efficient metal ion sensors [36–38]. Figure 3 shows the PL emission spectra of the colloidal GQDs (with a fixed concentration of 20 μg ml$^{-1}$) as a function of varying Ag$^+$ concentration. It can be clearly seen that there was appreciable quenching of the PL intensity as a function increasing of Ag$^+$ concentration. A small shift of ~5 nm in the maxima of the PL profile was also observed. This clearly indicated that Ag$^+$ incorporation was modifying the charge transfer mechanism of GQDs, which is a desired characteristic in sensing elements.

Figure 4. (a) PL spectra of GQDs and NGQDs. (b) Emission spectra of GQDs and DNA functionalized GQDs.

Figure 5. Zeta Potential variation in various samples.
The limit of detection was estimated using the Stern–Volmer equation [39]:

$$\frac{F_0}{F} = K_{SV}[C] + 1$$

(2)

where $F_0$ and $F$ are the fluorescence intensities recorded in the absence and presence of $Ag^+$, respectively, $[C]$ denotes the concentration of $Ag^+$ and $K_{SV}$ is the Stern–Volmer quenching constant. Two different linear regions were observed, in the Stern–Volmer plot shown in figures 3(a) and (b). The quenching mechanism showed contributions from both dynamic and static processes. This could be inferred from the non-linear curves of the Stern–Volmer plots. Using these linear regions, the limit of detection was estimated using the equation, $LOD = 3\sigma/S$ (for details see the supplementary information). The obtained numerical values are listed in table 1. These LOD values were low but similar to those reported earlier [40, 41].

The absorption spectrum of GQDs (see figure S6(a)) showed two dominant peaks, one at 230 nm and the other near 350 nm. The origin of such UV absorption peaks has been attributed to $\pi \rightarrow \pi^*$ transition of the C=C bonds [42]. In the present case, the quantum yield ($\sim73\%$) of GQDs was estimated using equation (1) and the band gap of $\sim2.91$ eV was obtained by fitting the Tauc equation to the UV spectra (see figure S6(b)). Methylene blue was used as the reference ($QY = 52\%$, see table S1).

As mentioned earlier, doping with hetero-atom can steadily enhance the intrinsic properties of carbon based materials. NGQDs have been found to show distinctive var-

iations in confinement, edge and surface states [43]. This can lead to improvement in the optical properties. The PL spectrum of NGQDs, shown in figure 4(a), clearly indicate towards higher emission intensities, in comparison to that observed in GQDs. The limit of $Ag^+$ detection observed using NGQDs (see figures 3(c) and (d)) were lower than that estimated in GQDs. The corresponding quantum yield obtained was more than 88%.

Quite interestingly, there have been few reports that deal with the interaction studies of DNA with bio-hazardous silver [44, 45]. DNA consists of deoxy-ribose sugar, nitrogenous bases (purine: adenine(A) and guanine(G); pyrimidine: thymine(T) and cytosine(C)) and phosphate [46]. The nitrogenous bases of DNA can also act as a source of nitrogen. The question, which immediately became evident was: whether

![Figure 6](attachment:image.png)

Figure 6. (a) PL Intensity profile for $Ag^+$ detection of CT/GQDs. (b) PL Intensity profile for $Ag^+$ detection of pET28a/GQDs. (c) Stern–Volmer curve fitting of CT/GQDs. (d) Stern–Volmer curve fitting of pET28a/GQDs.
the use of DNA will lead to improvement in Ag\(^+\) sensing and nitrogen doping of GQDs? For confidently answering this, two different types of DNA viz, pET28a (supercoiled form, 5.3 kb) and calf thymus (CT, linear form, 3 kb) were used to carry out a series of experiments. The choice of these two DNAs were driven by the known fact that they have varying percentages of nitrogen [47]. CT and pET-28a DNA have approximately 42% and 53% G≡C (Guanine, Cytosine) base pairs and 58% and 47% A\(\rightarrow\)T (Adenine, Thymine) base pairs, respectively.

Initially, to evaluate the extent of surface charge modification occurring in GQDs, with the addition of DNA, zeta potential variation was measured. The encircled data points shown in figure 5 depict the varying magnitude of the zeta potential in DNA functionalized GQDs. Modulation of zeta potentials clearly indicated towards the adsorption and interaction of DNA with the GQD surfaces. In addition to Ag\(^+\), further change in the zeta potential was observed (see table S2). This gave ample evidence that the DNA functionalized GQDs can be used as a metal ion sensor.

The PL emission spectra of DNA functionalized GQDs, in aqueous solution, were recorded at room temperature using a 300 nm excitation wavelength from a 525 nm laser. GQDs showed strong green PL under UV light (see figure S7 of SI). The PL spectrum for the different conjugates is shown in figure 4(b). The observed emission peaks, in two different DNA was red shifted and showed increase in the emission intensities. This suggested improvement in the charge transfer mechanism, due to the interaction of DNA with GQD surfaces. This can occur because of the nucleobases induced \(\pi-\pi\) stacking [48]. It has been seen that the nitrogenous base can also create edge effects in the GQDs layers causing enormous increase in the PL intensity. The stability of the GQD was confirmed by measuring the PL intensity at different pH values, this is shown in figure S8.

Figure 6 shows the emission spectra of DNA functionalized GQDs, with varying concentrations of Ag\(^+\). In both the cases, appreciable reduction in the intensity was discernible, which indicated Ag\(^+\) sensing. It was immediately clear that the sensing was much higher than that observed in pure GQDs, NGQDs or DNA alone. This proved the hypothesis that two sensing elements viz., GQD and DNA was synergistically combining and ensuring enhanced and efficient sensing of Ag\(^+\). The limit of detection is tabulated in table 1. The limit of detection of Ag\(^+\) for DNA functionalized GQDs were higher than those recommended by the US Environmental Protection Agency [49]. pET-28a functionalized GQDs started to quench the emission signal when 0.05 nM Ag\(^+\) was added. In comparison, for the CT functionalized GQDs, the value was 0.02 nM. Values lower than these did not show significant quenching of PL emission spectra.

The observation can be explained in terms of the Förster resonance energy transfer (FRET) process, which describes the energy transfer between two light-sensitive molecules. In this case, GQDs and DNA would act as the two fluorophore molecules. Chemically produced QDs have specific ligands or receptors, which allow the well defined nature of charge transfer process when an analyte interacts with its surface [50]. This leads to a dipole–dipole type interaction, whose magnitude depends on the separation distance \((1/r^6)\), where \(r\) is the Förster distance) between the two fluorophores.

The FRET efficiency (E) [51] can be obtained using the equation given below:

\[
E = \frac{R_0^6}{R_0^6 + r^6} \tag{3}
\]

where \(R_0\) is the Förster radius [42]. The transition metal can lead to dynamic quenching of the fluorescence signal [52]. The ability of metal to quench the fluorescence signal depends on its charge density and plasmonic nature. It was found that metals with smaller charge densities have larger quenching efficiencies [53]. In the present case, Ag\(^+\) acts as an analyte that forms a cytosine-Ag-cytosine complex structure [54].

As this complex structure changes in size, the magnitude of quenching also varies accordingly. Therefore, the quenching of PL intensity shown in figures 6(a) and (c) can be attributed to a strong FRET signal. The affinity between DNA functionalized GQDs to Ag\(^+\) depends on the concentration of nitrogenous bases [55, 56]. These nitrogenous bases will be...
adsorbed by the graphene substrate (shown in scheme 1). The content of C and G are higher in pET28a than in DNA of CT. The binding strength of Ag⁺ to the nitrogenous bases follows C > G > A > T, as reported earlier. The structures of these two DNA are different: pET28a is supercoiled whereas CT is linear, though their sizes are very close (evident from the measurement of the radius of gyration, Rg). Therefore, surface contact of GQDs with pET28a is slightly lower than that observed in CT. The major and minor grooves of DNA provides extra sites (less for pET28a) where these metal can bind along with GQDs [57]. As a result, the limit of detection was higher for CT modified GQDs than pET28a.

5. Conclusion

DNA functionalized graphene quantum dots can lead to high performing sensing systems for Ag⁺ detection. It is established that DNA can also be used for doping carbon based structures with hetero-atom such as N. The synthesized GQDs work as fluorophore, while the Ag⁺ acts as a fluorescence quencher. The observed energy transfer mechanism clearly indicates that GQDs/NGQDs can work as a fluorescent sensing probe for label-free detection of Ag⁺, with high sensitivity and selectivity. The limit of detection, found in the range of 0.05 to 0.02 nM, was lower than that recommended by the US Environmental Protection Agency. Such studies will make this system extremely useful for large scale industrial applications.

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